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The SOS response of *Escherichia coli* is the prototypic DNA damage repair and cell cycle control system, analogous to checkpoint control in eukaryotes. The SOS response includes blocking the cell cycle, global mutagenesis via more than one mechanism, and up regulation of DNA repair and recombination functions. Adaptive mutation is a mutational program in non-growing cells subjected to starvation and so is also a temporary mutagenic response to environmental stress. It entails global hypermutation, and previously, the signal transduction pathway from the environment to the DNA was unknown. Previously, we demonstrated that adaptive mutation of a *lac* allele in *E. coli* is under control of the SOS response, and that SOS induced levels of component(s) other than or in addition to RecA (hRad51 homologue) are also involved. We have demonstrated that the SOS inducible error-prone polymerase DinB/DNA pol IV is required for most adaptive mutation. DNA pol IV is not required for growth-dependent mutation (indicating that it is specific to adaptive mutation) and is not required for repair of UV or oxidative damage lesions. All this is consistent with a role for DNA pol IV for promotion of mutation in response to stress

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Introduction

My work focuses on the SOS response in Stressful Lifestyle Associated Mutation (SLAM). SLAM has a number of features distinct from growth-dependent mutation including that: (i) SLAM occurs in the absence of growth while starving; (ii) SLAM occurs in a hypermutable subset of the population; (iii) SLAM has a unique sequence spectrum, being -1 deletions at mononucleotide repeats whereas growth dependent Lac^+ reversions are heterogeneous; (iv) SLAM occurs in cells in which mismatch repair is limiting; and (v) SLAM requires homologous recombination proteins RecA, RecBC and RuvABC. The assay system that we use to study SLAM consists of *Escherichia coli* with a deletion of the *lac* region on the chromosome, and a *lacIΩZ* fusion carried on an F' conjugative plasmid. The *lac* fusion has a +1 frameshift in it, such that these cells are phenotypically Lac^-. These cells are plated on medium containing lactose as the sole carbon source. Lac^+ mutant colonies arise over time, and are counted each day.

Body

I am interested in understanding the SOS response, and how it is involved in SLAM. The SOS response is a DNA damage/cell cycle control response in *E. coli*. It works by sensing processed DNA damage (in the form of single-stranded DNA), which is coated by the protein RecA. This activates RecA, such that it acts as a co-protease to allow cleavage of several protein targets within the cell. These targets include the LexA repressor, the phage λ CI repressor and the UmuD translesion synthesis protein. LexA cleavage de-represses a regulon of at least 42 genes involved in DNA repair and recombination, cell division inhibition, and induced mutagenesis.

I have shown previously that efficient SLAM requires a functional SOS response because it requires LexA cleavage (McKenzie *et al.*, 2000). This indicates that induction of a Lex-repressed gene(s) is required for full levels of SLAM. One possible LexA-repressed candidate is the error-prone DNA polymerase, pol IV.

I have examined the role of pol IV, encoded by the *dinB* gene, in SLAM. Others have shown that pol IV is required for phage λ untargeted mutagenesis, and that its overproduction increases the spontaneous mutation frequency over 10-fold. -1 deletions at mononucleotide repeats are stimulated more than 800-fold. DNA pol IV is a prototype for a large, recently recognized superfamily of error-prone polymerases, the DinB/UmuDC superfamily, that has homologues in all 3 kingdoms of life. These include the XPV (xeroderma pigmentosum variant) polymerase, and several other human homologs whose functions are not known.

Pol IV is the first gene in an apparent operon with three genes of unknown function downstream. Thus, to study only its loss-of-function, I could not use either of the two existing null alleles which are almost certainly polar (one deletes part of the next gene and the other is a large phage Mu insertion). I constructed a site-directed mutation encoding a substitution of a highly conserved amino acid, the protein product of which was shown by others to be inactive as a polymerase *in vitro*, and not to promote mutation when overproduced *in vivo*. When I knocked-out DNA pol IV with this allele, I found that SLAM was reduced three to five fold. I was able to complement this defect in SLAM with the wild-type DNA pol IV gene placed at an ectopic site on the chromosome, with its natural promoter. This allowed us to conclude that pol IV is required for efficient SLAM. Further, I showed that 40 % of the Lac^+ colonies in a pol IV deficient strain were not actually Lac^+ mutants, but contained amplified arrays of the leaky Lac^- gene. This indicates that the phenotype of the DNA pol IV mutant is more dramatic than is immediately obvious. Mutation to Lac^+ is actually reduced to about 15% of that in an isogenic $polIV^+$ strain. Adaptive amplification is a pathway that occurs in $polIV^+$ cells, but at a low frequency (around 5-15% of Lac^+ colonies appearing on Day 5 are amplified rather than mutant). The amplification route to Lac^+ also appears to require homologous recombination proteins. Pol IV is the first protein required for SLAM that is not also required for amplification.

The requirement for pol IV led me to ask whether DNA pol IV was required for the characteristic sequence spectrum of SLAM, the –1 deletions at monocleotide repeats: Are the remaining Lac^+ mutants in a DNA pol IV mutant still -1 deletions at mononucleotide repeats or do they display a different sequence spectrum? I sequenced thirty-one day 5 Lac^+ mutants from the pol IV deficient strain, and found that the sequence spectrum is altered (see Figure 5, McKenzie et al., 2001). In the $pol\ IV^+$ strain, 2/3 of the Lac^+ mutations are at a particular hotspot repeat, with the rest distributed at a number of other mononucleotide repeats. In the DNA pol IV mutant, 2/3 of the mutations were at the hotspot, with only a single mutation seen at another mononucleotide run, and six other mutations (large deletions and insertions). We conclude that DNA pol IV promotes -1 deletions at a variety of mononucleotide repeat sites during SLAM. All of these results are now published in *Molecular Cell*. A copy of the manuscript will be provided.

We were curious as to which DNA polymerase is responsible for the SLAM that remains in a DNA pol IV mutant. With Phil Hastings, I have shown that the remaining mutations are accessible to correction by an anti-mutator DNA pol III (the house-keeping polymerase) mutant. This suggests at least two possible models: (i) that the additional errors are made normally by DNA pol III, (ii) the errors are made by another non-processive polymerase, and can be corrected by loading of DNA pol III or (iii) the errors

are made by another polymerase that the special antimutator pol III excludes from DNA, perhaps because it is more processive than the wild-type pol III enzyme.

We are interested in other functions of pol IV. One possibility is that DNA pol IV is used to incorporate bases across from damaged bases in the DNA (which is what many of the pol IV homologues do). I have looked for DNA damage survival phenotypes to test this idea, and so far, having looked at oxidative damage and UV survival, I have found no defect in survival in a DNA pol IV mutant. This is consistent with the idea that SLAM occurs on undamaged template and pol IV does make errors on undamaged template. I intend to examine further DNA damaging agents in hopes of revealing types of damage dealt with by polIV.

Knowing that polIV is required for SLAM provides insight into both SLAM and functions of polIV beyond SLAM. In one model for SLAM, a strand exchange intermediate primes error-prone DNA synthesis. Perhaps pol IV is required for priming DNA synthesis from genetic recombination intermediates. Our lab has excellent tools for testing this possibility, using phage lambda as a DNA substrate and conditions under which only recombination can give progeny. I am currently examining whether DNA pol IV is required for replication in genetic recombination intermediates.

I have also been involved in the training of 2 rotation students, in projects that are examining the role of transcription-coupled repair in SLAM and examining the nature of hypermutable subpopulations in SLAM. I am following up evidence that transcription coupled repair may have a role in SLAM. I have also been mentoring a high school, now undergraduate student since June 1999. His work is part of the aforementioned Mol Cell paper. He is currently helping me find if the open-reading frames downstream of *dinB* are indeed part of an operon, and what role they may play in mutation. Finally, I am first author on a review about pol IV and adaptive strategies in pathogens which is currently in press at Current Opinion in Microbiology (a copy of this manuscript is attached).

As an added note, due to the massive flooding in Houston in June of 2001, I have lost approximately one month of work (it still remains to be seen how much damage has been done). Baylor College of Medicine was closed and without power for two weeks, and all experiments underway at the time of the flood were destroyed, which has resulted in a setback of at least a month.

Key accomplishments (July 2000-July 2001)

- demonsrated that DNA polymerase IV is required for SLAM (published in *Molecular Cell*)
- mentored 3 students in projects dealing with mutation and recombination in *E. coli*
- wrote a review of SLAM and hypermutation in pathogenic bacteria for *Current Opinion in Microbiology* (submitted)

Reportable outcomes (July 2000-July 2001)

Meeting Presentations:

2000. Lost Pines Molecular Biology Conference. October 13-15. Lost Pines, Texas. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

2000. Molecular Genetics of Bacteria and Phages Meeting, August 22-28, Cold Spring Harbor Laboratory. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

Publications:

(July 2000-July 2001)

Bull, HJ, **GJ McKenzie**, PJ Hastings, & SM Rosenberg. 2000. Letter: The contribution of transiently hypermutable cells to mutation in stationary phase. *Genetics* 156: 925-926.

McKenzie, GJ, PL Lee, M-J Lombardo, PJ Hastings & SM Rosenberg. 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol Cell* 7: 571-579.

McKenzie, GJ & SM Rosenberg. 2001. Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. *Curr Opin Microbiol* (in press).

Conclusions

I have made substantial headway in understanding the role of the SOS response and DNA pol IV in SLAM. I have identified pol IV as the main polymerase required for SLAM and am currently working to further understand the role of DNA pol IV in mutation and DNA repair in *E. coli*.

Letter to the Editor

Response to John Cairns: The Contribution of Transiently Hypermutable Cells to Mutation in Stationary Phase

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In his letter, John Cairns reiterates the model described in his Appendix (CAIRNS 1999) to ROSCHE and FOSTER (1999) and seems to be concerned that we have not adopted it as an explanation for our observations (BULL *et al.* 2000). His model is that most Lac$^+$ adaptive mutants arise in one transiently mutating cell population while concurrently, secondary (unselected) mutations, and a smaller number of Lac$^+$ adaptive mutants associated with them, arise in a different transiently hypermutating subpopulation. The two populations were proposed to produce mutations via different mechanisms, using different gene products (ROSCHE and FOSTER 1999).

In fact, we did not reject a multiple-population model (BULL *et al.* 2000). We favor the simpler model of a single transiently mutable subpopulation producing both Lac$^+$ and secondary mutations (HALL 1990; TORKELSON *et al.* 1997) partly because the data that can be used to distinguish between the two models are very sparse such that other interpretations of those data remain possible. In the absence of more extensive data, a simpler model is more attractive.

We also favor the single subpopulation model because the data presented by BULL *et al.* (2000) imply a recombinational mechanism of chromosomal secondary mutation, as is found for all Lac$^+$ adaptive mutation (HARRIS *et al.* 1994). This contradicts the proposal that one population produces most Lac$^+$ recombinationally, but that chromosomal secondary mutations (and the Lac$^+$ associated with them) arise in a separate subpopulation that is mutating via a different mechanism.

Cairns' principal criticism is the use of a Poisson distribution when individual events have widely different mutation rates. We did not calculate a Poisson distribution based on mutation frequencies that covered an ~50-fold range. When TORKELSON *et al.* (1997) made this calculation, the very low frequency for mutation to fructose nonutilization (Fru$^-$) was omitted. We know now that the aberrantly low mutation frequency observed was caused by an inability of most Fru$^-$ cells to utilize lactose (see FRAENKEL 1996), such that Fru$^-$ cells Lac$^+$ are counterselected. The remaining frequencies show a range of only fourfold. The appropriate numbers from TORKELSON *et al.* (1997) are 286 double mutants (Lac$^+$ plus one secondary mutation) and 5 triple mutants (Lac$^+$ plus two secondary mutations) among ~42,000 Lac$^+$ mutants. These numbers omit Experiment 1, Table 2 of TORKELSON *et al.* (1997), because only 5-fluorocytosine (5-FC) resistance was scored in that experiment. Also omitted is the quadruple mutant (Lac$^+$ plus three other mutations) and four of the double mutants, because these isolates have a stable mutator phenotype. Both the size of the subpopulation and the mutation rates within that population are unknown initially. We assume that an average mutation rate can be applied to the remaining targets. The mutation rate to Lac$^+$ (4 Lac$^+$ per 850 Tet^R or 4.7×10^{-3}; FOSTER 1997) can be seen to be comparable to that of the remaining targets (3×10^{-3}–0.7×10^{-3} secondary mutants per Lac$^+$; TORKELSON *et al.* 1997). A reasonable fit to a Poisson distribution can be obtained by using an aggregate mutation rate for the five targets other than *lac* of 0.007 mutations/cell/4 days (the length of time over which these mutants formed under starvation). At this mutation rate, we expect 292 Lac$^+$ isolates to have one other mutation, and 1 to have two other mutations among 42,000 Lac$^+$ isolates. (Compare this with 286 and 5 observed in the same two classes.) Given that the individual mutation rate to Lac$^+$ would be about one-fifth of the aggregate mutation rate, the hypermutating subpopulation from which they arose would be 3×10^7 cells (42,000 mutations per 4 days ÷ 0.0014 mutations per hypermutating cell per 4 days). With the 42,000 Lac$^+$ being ~10^{-6} of all cells (4.2×10^{10}), the frequency of hypermutating cells would be 7.1×10^{-4} of the whole population. These numbers are revised from those esti-

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mated by TORKELSON *et al.* (1997), in which we mistakenly applied the aggregate mutation rate (rather than one-fifth of the aggregate) to Lac^+, and thereby arrived at a 10-fold smaller subpopulation and 2-fold higher mutation rate. The net conclusion does not differ.

We agree with Cairns that an excess of observed triple mutants relative to expected would argue in favor of multiple populations. However, on the basis of the current scant data, we cannot take the observed numbers as showing a significant deviation from the expectation, though further data might perhaps do so. Specifically, we are not persuaded that the 1 triple expected (per 292 doubles) deviates significantly from the 5 observed (per 286 doubles). This means that, on the basis of the data of Torkelson *et al.*, we do not reject the simpler hypothesis of a single transiently hypermutating subpopulation giving rise to all Lac^+ and the secondary mutations.

The data of ROSCHE and FOSTER (1999) cited by Cairns are very interesting. Although the relevant numbers in that work are larger than those discussed here, we feel that more are needed to discriminate between single- and multiple-subpopulation models. Because the simpler model of one subpopulation is also more harmonious with our observation of recombination-promoted mechanisms for both secondary (BULL *et al.* 2000) and all Lac^+ mutations (HARRIS *et al.* 1994), it seems the more economical model at present. Investigating the mechanism of the unselected hypermutation (*e.g.*, BULL *et al.* 2000) should be an effective tactic for addressing the key issue here: Is Lac^+ adaptive mutation a process that generates only adaptive mutations, or is the observed, concurrent chromosomal hypermutation part of the same process?

We are indebted to Russ Maurer for advice on the mathematics. This work is supported by National Institutes of Health grants R01-GM53158 and R01-AI43917.

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SOS Mutator DNA Polymerase IV Functions in Adaptive Mutation and Not Adaptive Amplification

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Summary

Adaptive point mutation and amplification are induced responses to environmental stress, promoting genetic changes that can enhance survival. A specialized adaptive mutation mechanism has been documented in one *Escherichia coli* assay, but its enzymatic basis remained unclear. We report that the SOS-inducible, error-prone DNA polymerase (pol) IV, encoded by *dinB*, is required for adaptive point mutation in the *E. coli lac* operon. A nonpolar *dinB* mutation reduces adaptive mutation frequencies by 85% but does not affect adaptive amplification, growth-dependent mutation, or survival after oxidative or UV damage. We show that pol IV, together with the major replicase, pol III, can account for all adaptive point mutations at *lac*. The results identify a role for pol IV in inducible genetic change.

Introduction

Radman (1975), Echols (1981), and others have suggested that states of accelerated evolution might be induced in response to stress and that enzymes might be specialized for this purpose. The discoveries of adaptive point mutation in bacteria and yeast, and of adaptive amplification in bacteria (Hastings et al., 2000), support the idea of differentiated states of hastened genetic change (reviewed by Rosenberg, 2001). Adaptive mutation is a process of increased mutability that occurs in stationary phase starving cells and can confer mutations allowing survival. There are many assay systems for its study (reviewed in Rosenberg, 1997, 2001; Foster, 1999), but in only one so far has adaptive mutation been demonstrated to occur by a molecular mechanism different from spontaneous mutation in growing cells (and so to be a separate process). That assay measures reversion of a *lac* +1 frameshift allele carried on an F′ episome in *Escherichia coli* (Cairns and Foster, 1991). In the *lac* system, one distinct mechanism produces adaptive point mutations, conferring a Lac$^+$ phenotype via compensatory frameshift mutations. Also in the *lac* system, a separate adaptive response produces adaptive amplifications (Hastings et al., 2000, and references therein for previous studies of amplification in bacteria). In adaptive amplification, the leaky *lac* mutant gene is amplified to many copies such that sufficient β-galactosidase activity is produced for growth on lactose medium *without* acquisition of a Lac$^+$ point mutation. Adaptive point mutation and amplification are separate adaptive responses and are both different from Lac$^+$ mutation in growing cells.

The adaptive point mutation mechanism at *lac* can be summarized as follows. The adaptive mutations occur after exposure to lactose medium (McKenzie et al., 1998) and require homologous recombination proteins of the RecBCD double strand break repair (DSBR) system (Harris et al., 1994, 1996; Foster et al., 1996). DSBR is proposed to promote mutation by priming replication during which DNA polymerase errors occur (Harris et al., 1994). Whereas growth-dependent Lac$^+$ mutations are heterogeneous, the adaptive mutations are nearly all −1 deletions in small mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg et al., 1994), resembling DNA polymerase errors formed by a template slippage mechanism (Streisinger et al., 1966; reviewed in Ripley, 1990). The adaptive mutations accumulate during a transient period of mismatch repair protein deficiency during starvation (Longerich et al., 1995; Harris et al., 1997b, 1999). The adaptive mutants, once formed, bear high frequencies of unrelated mutations throughout their genomes, indicating that some or all of the adaptive mutants arise during a transient genome-wide hypermutability (Torkelson et al., 1997; Rosche and Foster, 1999; Bull et al., 2000a; Godoy et al., 2000; and see Bull et al., 2000b; Cairns, 2000 for further discussion). Finally, efficient recombination-dependent adaptive mutation requires a functional SOS response for upregulation of a protein(s) other than or in addition to RecA (McKenzie et al., 2000). One infers that both recombination and SOS are required because recombination genes are required that are not also required for an SOS response (Foster et al., 1996; Harris et al., 1996).

The enzymatic basis of the mutability underlying adaptive mutation at *lac* has not been elucidated fully. Either of two different (general) mechanisms seems possible. On the one hand, the postreplicative mismatch repair (MMR) system (reviewed by Modrich and Lahue, 1996) becomes limiting transiently during adaptive mutation (Harris et al., 1997b, 1999), and genetic evidence implicates the major replicative DNA polymerase, pol III, in adaptive mutation (Foster et al., 1995; Harris et al., 1997a). Therefore, a normal rate of DNA polymerase error could lead to mutability because of failure to correct those errors. On the other hand, the involvement of the SOS response suggests (among other possibilities) that special mutator enzymes controlled by SOS could be responsible (McKenzie et al., 2000). The *umuDC*-encoded mutator DNA polymerase (pol) V is not required (Cairns and Foster, 1991; McKenzie et al., 2000). This study examines the other SOS mutator polymerase, pol IV, encoded by *dinB*.

Pol IV is a poorly processive error-prone DNA polymerase (Wagner et al., 1999; but see Tang et al., 2000; Wagner et al., 2000) and a member of the large, newly elaborated DinB/UmuDC superfamily of DNA polymerases in bacteria, archaea, and eukaryotes (reviewed by

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Friedberg et al., 2000). The discoveries of multiple DNA polymerases in all living organisms have raised the question of why cells have so many (e.g., five are known currently in *E. coli*). What are their functions? Some of the DinB/UmuDC polymerases are translesion polymerases known to promote DNA damage survival by allowing replication to bypass otherwise replication-blocking lesions. The human XP-V (xeroderma pigmentosum variant) tumor suppressor protein (of the Rad30 subfamily) and *E. coli* pol V (of the UmuDC subfamily) are examples. However, the function(s) of pol IV (DinB subfamily) and three of its mammalian homologs (Friedberg et al., 2000) have been elusive. Pol IV may participate in mutation of undamaged phage λ DNA during infection of irradiated *E. coli* (λ untargeted mutagenesis; Brotcorne-Lannoye and Maenhaut-Michel, 1986). Pol IV overproduction causes hypermutation including −1 frameshifts and some substitutions (Kim et al., 1997; Wagner and Nohmi, 2000). The purified pol IV enzyme makes similar errors (Wagner et al., 2000).

We shall report that pol IV is required for most adaptive point mutation at *lac*, but not for mutations in growing cells, survival of UV or oxidative damage, or adaptive amplification. Thus, one function of pol IV in *E. coli* involves environmentally inducible genetic change.

Results

Experimental Strategy

To test whether adaptive mutation occurs in cells lacking a functional DNA pol IV, encoded by *dinB*, we constructed isogenic *dinB*$^+$ and mutant strains. *dinB* is the first gene in an apparent operon of four damage-inducible (Courcelle et al., 2001) SOS genes: *dinB*, *yafN*, *yafO*, and *yafP*. The *yaf* genes have unknown functions, though YafN has homology to the anti-toxin of the *relBE* operon (Grønlund and Gerdes, 1999). All of these genes are likely to be inactivated by previously published null alleles of *dinB*: a deletion of *dinB* and part of *yafN* (Kim et al., 1997), and an insertion (Kenyon and Walker, 1980). To remove only pol IV function, we created a nonpolar null allele of *dinB* identical to *dinB10* (Wagner et al., 1999), which replaces a highly conserved amino acid (R49F), producing a mutant polymerase that is inactive in vitro and does not enhance mutation when overproduced in vivo. The *lac* frameshift–bearing strain carries two copies of the *dinB*$^+$ gene, one on the F′ and one in the chromosome (Experimental Procedures). We constructed strains carrying *dinB10* at both sites.

In adaptive mutation assays, Lac$^-$ cells are plated onto lactose medium and incubated for several days (Experimental Procedures). Lac$^+$ mutant colonies that appear early (about day 2) represent growth-dependent mutants formed before plating on lactose medium (Cairns and Foster, 1991; see Harris et al., 1999). Colonies that appear late (e.g., day 3–7) consist of a majority of adaptive point mutants and a minority of adaptive amplified clones, both formed after plating on lactose medium (McKenzie et al., 1998; Hastings et al., 2000).

Pol IV Is Required Specifically for Adaptive Point Mutation at *lac*

Replacement of both copies of *dinB*$^+$ with *dinB10* reduces adaptive mutation about 4-fold (Figure 1A), indicating that DNA pol IV function is required for most adaptive mutation in the *lac* system. This phenotype can be complemented with a single, ectopic chromosomal copy of *dinB*$^+$ (Figure 1B), indicating that the decrease in adaptive mutation is caused solely by the loss of pol IV, and not other genes in the putative *dinB* operon. We note that a single chromosomal copy of *dinB*$^+$ is sufficient for adaptive mutation at *lac* (Figure 1B), contrary to the suggestion that expression of the extra copy of *dinB* on the F′ might be required (Godoy et al., 2000). These results indicate a biological role for pol IV: it promotes adaptive mutation.

The amount of adaptive point mutation requiring pol IV is greater than is apparent from the total colony counts in Figure 1. About 42% of the day 5 (i.e., adaptive) Lac$^+$ colonies that remain in the pol IV-deficient strain carry amplified arrays of the leaky *lac*$^-$ allele rather than a point mutation, as compared with 9.5% for *dinB*$^+$ (Figure 1A). These classes were distinguished by their colony color after purification by streaking for single colonies onto rich X-gal medium (Experimental Procedures). The fact that amplified clones are about 40% of day 5 colonies in pol IV-deficient cells indicates that the reduction in adaptive point mutation in pol IV-deficient cells is actually about 85% (25% Lac$^+$ mutants seen, 60% of which are point mutants, leaves 15% point mutation remaining) (Figures 1A and 2B). Thus, the vast majority of the adaptive point mutation is pol IV dependent.

In addition, the data show that pol IV is not required for adaptive amplification. Amplified clones constitute ~10% of Lac$^+$ colonies in pol IV$^+$ cells (above) and ~40% of Lac$^+$ colonies in pol IV$^-$, in which the total number of Lac$^+$ colonies is reduced 4-fold (25% of that seen in pol IV$^+$). Thus, the number of amplified clones in pol IV$^-$ cells is approximately the same as in pol IV$^+$ (40% amplified of 25% total colonies equals 10%). Pol IV is therefore required specifically for adaptive point mutation and not for adaptive amplification.

To test whether pol IV is also required for growth-dependent mutation, we measured the mutation rate in *dinB*$^+$ and *dinB*$^-$ growing cells using fluctuation tests (in which mutant frequencies determined in multiple independent cultures are used to calculate rates; Experimental Procedures). To exclude adaptive mutants from the counts of growth-dependent Lac$^+$ mutants, we acquired ten independent Lac$^+$ mutant derivatives of the *dinB*$^+$ and *dinB10* strain. These were seeded at a known number of cells per plate onto lactose plates under exact experimental conditions, in parallel with the cultures in which growth-dependent mutants were being enumerated. These controls indicate the earliest possible time to count Lac$^+$ colonies for each cell genotype (the time at which the seeded Lac$^+$ control colonies become visible) (Harris et al., 1999). Failure to use these controls can give uninterpretable results, because both growth-dependent and adaptive mutants contribute to the colony counts from which mutation rates are calculated (Harris et al., 1994, 1996, 1997b, 1999). The results in Table 1 show that pol IV is not required for growth-dependent mutation at *lac*.

We find that pol IV mutation does not affect the rate of other growth-dependent mutations, including substitutions, frameshifts, and other mutations in growing

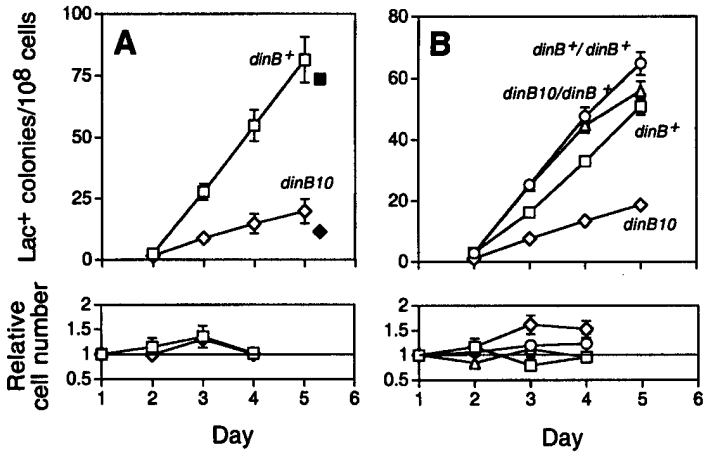

Figure 1. DNA Polymerase IV Is Required for Most Lac$^+$ Adaptive Mutation

(A) Total Lac$^+$ colonies are shown as open symbols. Lac$^+$ point mutants (see text) are plotted as filled symbols offset slightly from the day 5 point for clarity. The fraction of day 5 colonies carrying amplification (Experimental Procedures) was 9.5% (mean ± 2.6% SEM) in the *dinB*$^+$ and 42% (± 5.6%) in the *dinB10* strain.

(B) Decrease in mutation is complemented by a single, ectopic, chromosomal copy of *dinB*$^+$ controlled by its natural promoter. *dinB*$^+$ (open squares), *dinB10* (open diamonds), *dinB*$^+$ Δ*attB*::*dinB*$^+$ (open circles), and (Δ) *dinB10* Δ*attB*::*dinB*$^+$ (open triangles) strains SMR4562, SMR5830, SMR5834, and SMR5851, respectively. Means ± SEM (error bars) of ten independent cultures tested are shown (except for the filled symbols, mean ± SEM of four cultures). Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

cells (Figure 3). We conclude that pol IV is required specifically for adaptive mutation.

Our results disagree with a previous study, in which a *dinB* mutation appeared to decrease the rate of growth-dependent Lac$^+$ mutation slightly (Strauss et al., 2000). The reason for the difference may be that the earlier study did not account for adaptive mutations. Alternatively, the small rate change may have been due to the use of a polar *dinB* allele, which also disrupted genes downstream of *dinB*.

Pol IV is also not required for survival of UV irradiation and oxidative damage caused by hydrogen peroxide. As seen in Figure 4, the *dinB10* mutant is indistinguishable from an isogenic *dinB*$^+$ strain in UV survival and hydrogen peroxide resistance. Control isogenic strains carrying the *lexA3*(Ind$^-$) mutation, blocking SOS gene induction, or a mutation in *xthA*, encoding an exonuclease required for repair of peroxide-induced damage (Demple et al., 1983), show reduced resistance, as expected.

SOS/LexA Induction Promotes Adaptive Point Mutation Wholly via Pol IV

Because pol IV is one of the genes induced by the SOS response (reviewed by Walker, 1996), we asked whether pol IV alone can account for the requirement for SOS induction in adaptive point mutation (Cairns and Foster, 1991; McKenzie et al., 2000; Figure 2A). If induction of additional SOS-induced genes were required, then *dinB10 lexA3*(Ind$^-$) cells (SOS noninducible due to an uncleavable mutant LexA repressor) should produce fewer adaptive mutations than *dinB10* cells. However, our experiments showed that the rate of adaptive mutation in both genetic backgrounds is the same (Figure 2B), implying that induction of SOS genes that act independently of pol IV is not required. Thus, genes such

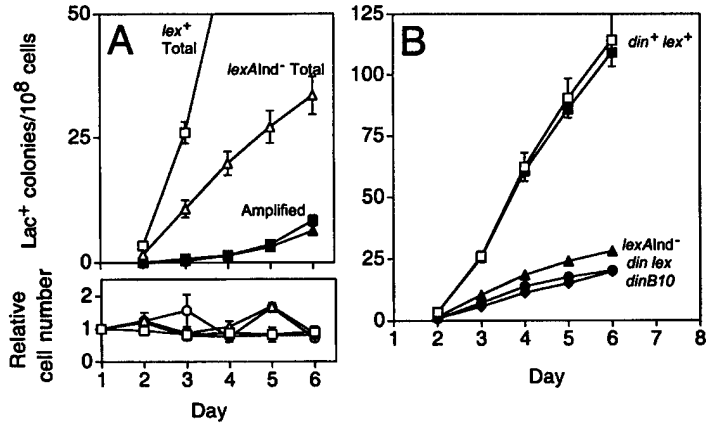

Figure 2. Different Roles of SOS Induction in Adaptive Amplification and Point Mutation

(A) Induction of the SOS/LexA regulon is not required for adaptive amplification. Total adaptive Lac$^+$ colonies (open symbols) are decreased by the *lexA3*(Ind$^-$) allele (open triangles), whereas the fraction amplified (filled symbols) is not. *lexA*$^+$ (squares) and *lexA3*(Ind$^-$) (triangles) strains SMR583 and SMR820, respectively.

(B) The contribution of SOS/LexA induction to adaptive point mutation is wholly via pol IV. Closed symbols display adaptive Lac$^+$ point mutants for *dinB*$^+$ *lexA*$^+$ (squares), *lexA3*(Ind$^-$) (triangles), *dinB10* (diamonds), and *dinB10 lexA3*(Ind$^-$) (circles) strains SMR583, SMR820, SMR5849, and SMR5850, respectively. This is the same experiment shown in (A) but with data from more of the strains tested in parallel shown, and point mutation displayed. Both sets of experiments were performed three times with similar results. In (B), the total adaptive Lac$^+$ colonies are also shown for the *dinB*$^+$ *lexA*$^+$ control strain (open squares). Means ± SEM (error bars) of ten independent cultures tested are shown (except for the filled symbols, mean ± SEM of four cultures). Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

Table 1. DNA Polymerase IV Does Not Affect *lac* Frameshift Reversion in Growing Cells

Relevant Genotype	Experiment	Median Number of Mutants	Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)	Mean (± SEM)
dinB$^+$	1	3.5	3.1×10^{-9}	$1.6 (\pm 0.3) \times 10^{-9}$
	2	12.8	1.9×10^{-9}	
	3	5.1	1.5×10^{-9}	
	4	5.0	1.8×10^{-9}	
dinB10	1	2.0	4.5×10^{-9}	$1.2 (\pm 0.3) \times 10^{-9}$
	2	7.4	1.2×10^{-9}	
	3	2.9	1.3×10^{-9}	
	4	3.0	1.1×10^{-9}	

Strains are *dinB*$^+$, SMR4562 and *dinB10*, SMR5830. See Experimental Procedures.

as the *recA*, *ruvA*, and *ruvB* recombination genes, which are required for adaptive mutation, appear to suffice at their noninduced (constitutive) levels. These results suggest that the requirement for SOS induction in adaptive point mutation (Cairns and Foster, 1991; McKenzie et al., 2000; Figure 2A) may be accounted for solely by pol IV.

Induction of LexA/SOS Genes Is Not Required for Adaptive Amplification

The SOS response was previously shown to be required for adaptive point mutation. We tested whether SOS-induced genes are also required for adaptive amplification. We found that blocking induction of the SOS/LexA regulon with the *lexA3*(Ind$^-$) allele (encoding an uncleavable LexA repressor protein; Mount et al., 1972; Lin and Little, 1989) decreases only point mutation, not adaptive amplification (Figure 2A, filled symbols). Thus, only adaptive point mutation, and not adaptive amplification, requires induction of LexA controlled genes, supporting the conclusion that these are separate pathways.

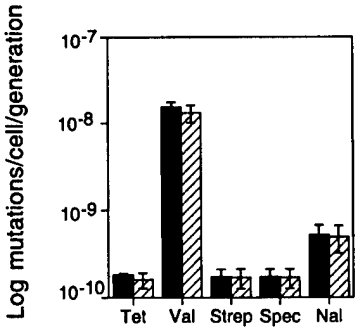

Figure 3. Rates of Frameshift and Substitution Mutations in *dinB*$^+$ and *dinB10* Cells during Growth

The various frameshift and substitution mutation assays (see LaRossa, 1996) follow: Val, a variety of different mutations in the isoleucine/valine biosynthesis genes, conferring valine resistance; Strep and Spec, substitution mutations in two ribosomal protein genes conferring streptomycin and spectinomycin resistance, respectively; Nal, substitution mutations in the *gyr* genes conferring nalidixic acid resistance; and Tet, reversion of a +1 frameshift mutation (4G to 5G, Experimental Procedures) in a chromosomal *tetA* gene conferring tetracycline resistance. This is similar to the 3G to 4G *lacI33* frameshift allele used in these adaptive mutation studies. *dinB*$^+$ (filled bars) and *dinB10* (hatched bars) strains are SMR4596 andSMR6049, respectively. Error bars, one SEM of three independent experiments.

Pol IV Contributes to –1 Deletions in a Variety of Mononucleotide Repeats

Lac$^+$ adaptive mutations are nearly all –1 deletions in small mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg et al., 1994). In the presence of wild-type *dinB*$^+$, most occur at a reversion hot spot (4Cs that include the +1 frameshift mutation inactivating *lac*), but a significant portion (about one-third) occurs at other mononucleotide repeats. We find that in the absence of pol IV, –1 frameshifts occur mostly at the hot spot (24/31 mutations sequenced, Figure 5), with other point mutations being larger insertions and deletions or not at mononucleotide repeats (Figure 5). The data imply that pol IV facilitates –1 deletions at many different mononucleotide repeats, mutations similar to the frameshift component of the error spectrum of the purified polymerase (Wagner et al., 1999).

Overlapping Roles of Pol III and Pol IV

Previous data suggested that pol III may play a role in adaptive point mutation. An antimutator pol III strain decreased the total number of adaptive Lac$^+$ mutations by about 4-fold (Foster et al., 1995; Harris et al., 1997a). In agreement with these results, we find that the antimutator pol III (encoded by *dnaE915*) reduces the number of adaptive point mutations by about 80% (Figure 6). Thus, neither pol IV mutation nor an antimutator pol III inhibits all adaptive point mutation. However, in cells carrying *dnaE915* and a defective pol IV (circles), adaptive point mutation is essentially abolished (Figure 6). These results show that the antimutator pol III decreases both the pol IV-dependent and the pol IV-independent adaptive point mutations, indicating overlapping roles for pol III and pol IV in this process (discussed below).

Discussion

The data presented in this paper imply that the SOS mutator DNA polymerase pol IV is a mutation-promoting enzyme required specifically for most (about 85% of) adaptive point mutation (Figure 1), but not for growth-dependent Lac$^+$ (Table 1) or other (Figure 3) mutation. Pol IV promotes adaptive mutations that are –1 deletions at a variety of mononucleotide repeats (Figure 5), similar to the frameshift component of the error spectrum of the purified enzyme (Wagner et al., 1999). Further, pol IV can account for the requirement for SOS induction in the *lac* system (Figure 2B, Cairns and Foster, 1991; McKenzie et al., 2000). Finally, pol IV is not required for

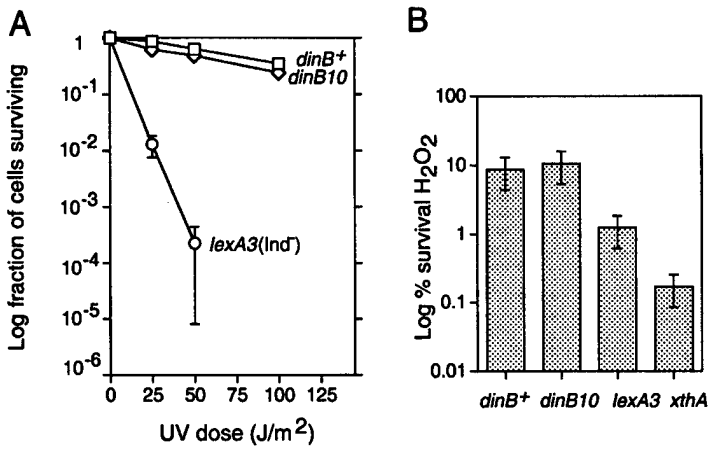

Figure 4. Loss of Pol IV Confers No Detectable Change in Survival of UV or Oxidative Damage

(A) UV sensitivity. Four cultures per strain were tested, and the means ± SEM (error bars) are shown. *DinB*$^+$ (open squares), *dinB10* (open diamonds), and *lexA3*(Ind$^-$) (open circles), strains SMR4562, SMR5830, and FC231, respectively.

(B) Sensitivity to hydrogen peroxide. Four cultures of each strain were tested in parallel, and the mean ± SEM are shown. Strains are as in (A) with the addition of SMR5287 lacking exonuclease III (encoded by *xthA*), used in base excision repair of oxidatively damage DNA (reviewed by Friedberg et al., 1995). Both experiments were performed three times with similar results.

resistance to UV light (Kenyon and Walker, 1980; Figure 4) or hydrogen peroxide (Figure 4).

Adaptive Amplification

The results also reveal that neither pol IV nor induction of SOS/LexA-controlled genes is required for adaptive amplification of *lac* (Figures 1 and 2A). These data add to the evidence that these two adaptive mechanisms are distinct by showing that they require different proteins. These data also suggest that the role of pol IV (and SOS induction) is in error-prone DNA synthesis that generates adaptive point mutations, but not generally in DNA synthesis in stationary phase, which would be expected to be required for both amplification and point mutation mechanisms.

Contributions of Pol IV and MMR Limitation to Mutability and the Characteristic Sequences of *lac* Adaptive Point Mutations

The requirement for an error-prone polymerase, pol IV, in adaptive point mutation supports models in which special error-prone synthesis leads to mutation, making previous models invoking depressed mismatch repair (MMR) as the sole basis of mutability implausible. However, limiting MMR also appears to contribute. First, apart from resembling the frameshift errors made by pol

Mutation	*dinB*$^+$	*dinB10*
-1 at hotspot mononucleotide repeat: nt 1039	22	24
-1 at other mononucleotide repeats	13	1
Other insertions and deletions	0	6
Total:	35	31

Figure 5. DNA Pol IV Promotes –1 Deletions at a Variety of Mononucleotide Repeat Sites in Lac$^+$ Adaptive Mutation

A roughly 300 nucleotide (nt) segment of DNA spanning the *lac* frameshift allele was sequenced from PCR-amplified DNA from day 5 *dinB10* Lac$^+$ point mutants (primers lacIL2 5′–AGGCTATTCTGGTGGCCGGA, and lacD2-GCCTCTTCGCTATTACGCCAGCT). Sequencing was performed by Lone Star Labs, Inc. (Houston, TX). Compensatory frameshift mutations in a possible 130 nt region between the two out-of-frame stop codons (boxed) can restore gene function. In *dinB*$^+$ cells, adaptive reversions are –1 deletions at a hot spot (nt 1039) and at many different mononucleotide repeats sites highlighted above (nt 1059, 1064, 1071, 1075, and 1109, data from Rosenberg et al. 1994). In *dinB10* cells, only the hot spot repeat is appreciably active for –1 repeat deletions, and other insertions and deletions are also prevalent. The other mutations include a –1 frameshift with an adjacent substitution (at nt 1094–5); a +2 insertion (nt 1092); an insertion of >40 bp (from 3′ of the sequenced area to nt 1120); and three large deletions of 103 bp (nt 1017–1119), 103 bp (979–1081), and 211 bp (nt 878–1088). Nt repeat positions are indicated above the leftmost base covered by the number, and the additional base of the original +1 frameshift mutation in the repeat at nt 1039 is not numbered.

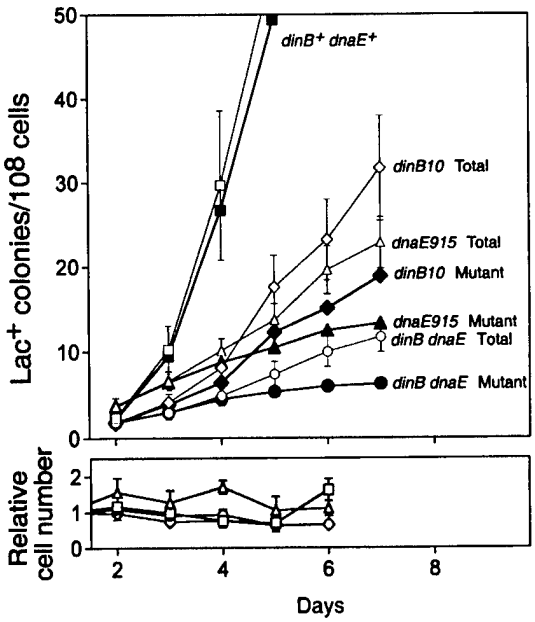

Figure 6. Overlapping Roles of Pol III and Pol IV in Adaptive Point Mutation

Open symbols are total Lac$^+$ colonies, and filled symbols point mutants only for strains carrying *dinB*$^+$ *dnaE*$^+$ (squares), *dinB10* (diamonds), *dnaE915* (triangles), and *dinB10 dnaE915* (circles): SMR6113, SMR5945, SMR6114, and SMR5944, respectively. The experiment was performed three times with similar results. Means ± SEM (error bars) of ten independent cultures tested are shown. Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

IV enzyme, the Lac$^+$ adaptive mutation sequences are identical to growth-dependent mutations in cells lacking postsynthesis MMR (Longerich et al., 1995). Second, MMR limitation has been demonstrated to occur, and to be required for, efficient adaptive mutation in this system (Harris et al., 1997b, 1999). Error-prone synthesis and limiting MMR are therefore both implicated and might possibly be related. For example, Wagner and Nohmi (2000) report that pol IV overproduction causes an insufficiency of MMR activity that can be alleviated by overproducing MutL. MutL also becomes limiting for MMR during adaptive mutation (Harris et al., 1997b, 1999) and in mutants with an error-prone DNA polymerase III (Schaaper and Radman, 1989). In all these cases, it could be that excess polymerase errors titrate MMR, causing a synergistic hypermutable condition. However, for Kim et al. (1997), pol IV overproduction did not produce a mutation spectrum similar to that of MMR mutant cells. This implies that MMR was not limiting in their overproduction experiments. Whether the demonstrated MMR limitation during adaptive mutation (Harris et al., 1997b, 1999) is caused by, or independently of, pol IV-produced errors, the combination is likely to interact synergistically to produce a condition of hypermutation.

Roles of Other DNA Polymerases

E. coli has five DNA polymerases. Pols II, III, and IV have been implicated in the synthesis during adaptive mutation as follows. First, the error-free SOS DNA pol II appears to compete with the polymerase(s) making adaptive mutations, in that pol II-deficiency increases adaptive mutation (Foster et al., 1995; Harris, 1997). Perhaps pol II competes with pol IV at the replisome. Second, an anti-mutator pol III allele decreases Lac$^+$ adaptive mutation ≥3-fold (Foster et al., 1995; Harris et al., 1997a), decreasing both pol IV-dependent and pol IV-independent point mutation (Figure 6). The apparent overlap between pol III and pol IV (Figure 6) can be understood by hypotheses in which pol III and pol IV compete with and/or substitute for each other on DNA (e.g., Friedberg et al., 2000; Tang et al., 2000). In one general model, pol IV makes the errors that become mutations. This is supported by the similarity of the sequence spectrum of adaptive mutations attributable to pol IV (Figure 5) with the frameshift error spectrum of the purified polymerase (Wagner et al. 1999). The pol III antimutator protein might exclude pol IV from DNA (and might then lower pol IV-independent point mutations by excluding some other polymerase). Alternatively, pol III might correct errors made by pol IV. It could also do both. In another general model, pol III could make errors that are fixed as mutations by pol IV (see Tang, et al. 2000). Other hypotheses are also possible. Whichever may be the case, the data indicate involvement of both polymerases and suggest that replisomes may exchange pols II (see above), III, and IV.

Function of Pol IV for *E. coli*

A biological function can now be assigned to pol IV, a member of the DinB branch of DinB/UmuDC superfamily polymerases, in adaptive mutation. Is this its only function? Other polymerases in the UmuDC, Rad30, and Rev1 branches of this superfamily are translesion polymerases (Friedberg et al., 2000), but the evidence for pol IV is ambiguous. Purified pol IV deals poorly with common UV lesions (Tang, et al. 2000), and pol IV-defective cells are not sensitive to UV (Kenyon and Walker, 1980, and Figure 4A) or hydrogen peroxide (Figure 4B). Although, together with pol V, pol IV was implicated in synthesis across benzo(a)pyrene adducts (Napolitano et al., 2000), that study used a deletion of *dinB* and part of *yafN* (probably also polar on *yafO* and *yafP*, see Experimental Strategy), making the conclusion uncertain. If translesion synthesis at adducts truly is a function of pol IV, it is a different role than the one pol IV plays in adaptive mutation because the former requires pol V (Napolitano et al., 2000), whereas the latter is pol V independent (Cairns and Foster, 1991; McKenzie et al., 2000). Pol IV might facilitate DNA replication promoted by DSBR recombination, the proposed source of replication in adaptive mutation (Harris et al., 1994). Yeast Rev3, or pol zeta (Rev1 subfamily), promotes substitution mutations associated with yeast DSBR (Holbeck and Strathern, 1997). Regardless of other possible functions of pol IV, its central role in adaptive mutability recalls suggestions of enzymes specialized for mutability (Radman, 1975; Echols, 1981, and others subsequently), accelerating evolution when needed.

Role of This Adaptive Mutation Mechanism in Bacterial Evolution

Frameshift mutations are usually thought of as inactivating genes. Is recombination-dependent adaptive muta-

tion generally relevant to bacterial evolution? First, in adaptive mutation at *lac*, substitutions probably also occur because overproduction of pol IV causes substitutions as well as frameshifts (Kim et al., 1997; Wagner and Nohmi, 2000). Second, many pathogenic bacteria regulate expression of "contingency genes" (used under stress) by frequent frameshift mutation events that turn gene functions off and on (e.g., Deitsch et al., 1997; Saunders et al., 2000). These bacteria might employ adaptive mutation strategies similar to those discussed here. In fact, the pathogens *Neisseria meningitidis* and *N. gonorrhoeae* have one or more genes homologous to *dinB* (open reading frame NMB1448 in strain MC58, Tettelin et al., 2000; and NMA1661 in strain Z2491, Parkhill et al., 2000). Third, regarding the relative importance of inducible mutation mechanisms, versus selection of preexisting mutator strains, we note that the mutator strains found among wild bacteria represent the minority (LeClerc et al., 1996; Matic et al., 1997; Denamur et al., 2000; Oliver et al., 2000). The majority of wild bacteria (80%–99%) are not mutators, such that adaptive mutation strategies may contribute appreciably (Rosenberg et al., 1998; Hastings et al., 2000).

Eukaryotic Homologs

Pol IV promotes inducible genetic change (above). Could its mammalian homologs function similarly? The mouse pol IV homologs, pol μ and pol λ, and true ortholog, DinB1 or pol κ (each also in humans), are abundant in lymphoid (μ) and germline cells (λ and κ), respectively (Friedberg et al., 2000). Their functions are unknown, although roles in somatic hypermutation (Friedberg et al., 2000) or other generation of diversity in immunoglobulin and/or T cell receptor genes seem possible. Could there be programmed mutation, driving evolution, in germ cells of mammals? As with the immune system, selections against deleterious mutations are stringent in germ cells (successful completion of development) such that programmed germline mutation/evolution might not be impossible.

Experimental Procedures

Bacterial Strains and Mutant Alleles

Bacterial strains used are isogenic to FC40 (Cairns and Foster, 1991, see also for FC231) and were constructed using standard P1 transduction methods (Miller, 1992). *dinB10* (Wagner et al., 1999) was constructed by PCR site-directed mutagenesis, replaced in the chromosome (Link et al., 1997) and transduced into a *proAB*$^+$ strain to link it with *proAB*$^+$. *proAB*$^+$ *dinB10* was transduced into the F′ replacing *proAB-81*::Tn*10*. The F$^-$ parent of FC40 (Cairns and Foster, 1991) was also transduced to carry *dinB10*, then mated with the F′ *lac* carrying *dinB10* to make the *dinB10* homozygous strain, SMR5830. *dinB10* was identified by (positive) DraI digestion of PCR products. Ectopic expression of *dinB*$^+$ in SMR5834 and SMR5851 was accomplished by replacement of the bacterial *attB* site with *dinB*$^+$ including its natural promoter (basepairs 249,092–255,436 of the *E. coli* genome sequence, as described; L. Gumbiner-Russo, M.-J. Lombardo, and S. M. Rosenberg, unpublished data). SMR583 (FC40 *malB*::Tn*9*), SMR820 (FC40 *malB*::Tn*9 lexA3*(Ind$^-$)), SMR5849 (SMR5830 *malB*::Tn*9*), and SMR5850 (SMR5830 *malB*::Tn*9*/*lexA3*(Ind$^-$)) carry *malB*::Tn*9* from D. Ennis (Lafayette, LA) and *lexA3*(Ind$^-$) from FC231 (Cairns and Foster, 1991). SMR5287 carries Δ(*xthA-pncA*)*90 zdi-201*::Tn*10* from BW9116 (*E. coli* Genetic Stock Center, Yale University). SMR6113 (FC40 *zae*::Tn*10dcam zae-502*::Tn*10*), SMR6114 (FC40 *zae*::Tn*10dcam dnaE915 zae-502*::Tn*10*), SMR5944 (SMR5830 *zae*::Tn*10dcam dnaE915 zae-502*::Tn*10*), and SMR5945 (SMR5830 *zae*::Tn*10dcam zae-502*::Tn*10*) carry alleles from NR9915 and NR9918 (Fijalkowska et al., 1993). SMR4576 and SMR6049 carrying *upp*::Tn*10dtet+1* (with a 4G to 5G frameshift at bp 331 of *tetA*; Foster, 1997) are described by H. J. Bull, M.-J. Lombardo, and S. M. Rosenberg (unpublished data).

Mutation and Amplification Assays

Adaptive mutation experiments were performed as described (Harris et al., 1996). Daily measurements of viable *lac*$^-$ cells on the plates (Harris et al., 1996) showed no net growth or death during the experiments. Growth-dependent Lac$^+$ mutation measurements used 40 tube fluctuation tests, as described (Harris et al., 1999). Mutation rates were calculated by the method of the median (Lea and Coulson, 1949; as modified by von Borstel, 1978). Other mutations rate assays used 30 tube fluctuation tests with Tet^R, Val^R, and Nal^R calculated by the method of the median and Strep^R and Spec^R by the P_0 method (Lea and Coulson, 1949; von Borstel, 1978; correction for P_0 as per Rosche and Foster, 2000). Because Tet^R colonies continue to appear over time, Tet^R assays were done with Tet^R controls as described for Lac (Harris et al., 1999, Results), to exclude mutants formed on the Tet plates and were scored at 12 hr (90%–100% of the control colonies visible). Selection agents were tetracycline, 10 μg/ml; valine, 5 μg/ml; streptomycin, 100 μg/ml; spectinomycin, 100 μg/ml; and nalidixic acid, 10 μg/ml.

The fraction of Lac$^+$ colonies carrying amplification rather than point mutation was determined in *dinB*$^+$ and *dinB10* day 5 Lac$^+$ colonies (40 colonies/culture, four independent cultures) of each strain as previously described (Hastings et al., 2000) by picking and restreaking Lac$^+$ colonies to LBH X-gal rifampicin medium to test instability of the Lac$^+$ phenotype. Unstable Lac$^+$ carry roughly 30 copies of *lac*$^-$ amplified DNA in direct repeats of 7–40 kb (Hastings et al., 2000). This method was also used for Figures 2 and 6.

UV and Oxidative Damage Survival Assays

Diluted saturated cultures (four/strain) in LBH medium (e.g., Torkelson et al., 1997) were plated on LBH plates and irradiated in a Stratalinker (Stratagene, La Jolla, CA). Sensitivity to hydrogen peroxide (H_2O_2) was measured as described (Demple et al., 1983), splitting log phase LBH cultures, exposing half to 5.6 mM H_2O_2 (and half to H_2O_2-free control medium) for 15 min, and plating for viable cells.

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Erratum

In the March *Molecular Cell* article by McKenzie et al., "SOS Mutator DNA Polymerase IV Functions in Adaptive Mutation and Not Adaptive Amplification" (7, 571–579), Table 1 contained four incorrect numbers in the column "Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)". The mean mutation rates in the table were correct. The conclusions from this table and of the paper are not altered by this correction. The corrected Table 1 is printed below and will be corrected in the online version of the article.

Table 1. DNA Polymerase IV Does Not Affect *lac* Frameshift Reversion in Growing Cells

Relevant Genotype	Experiment	Median Number of Mutants	Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)	Mean (± SEM)
dinB$^+$	1	3.5	0.96×10^{-9}	$1.6 (\pm 0.3) \times 10^{-9}$
	2	12.8	2.3×10^{-9}	
	3	5.1	1.5×10^{-9}	
	4	5.0	1.8×10^{-9}	
dinB10	1	2.0	0.63×10^{-9}	$1.2 (\pm 0.3) \times 10^{-9}$
	2	7.4	1.9×10^{-9}	
	3	2.9	1.3×10^{-9}	
	4	3.0	1.1×10^{-9}	

Strains are *dinB*$^+$, SMR4562 and *dinB10*, SMR5830. See Experimental Procedures in the article.

Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens

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'Adaptive mutation' is a collection of stress responses promoting mutations, some of which are advantageous. This year, in *Escherichia coli*, adaptive gene amplification was documented, and a parallel adaptive point mutation mechanism was linked to the error-prone DNA polymerase, pol IV (DinB). We suggest that DinB homologues may contribute to adaptive strategies of pathogens.

Abbreviations

DSB double-strand break; **DSBR** double-strand-break repair; **DSE** double-strand end; **MMR** mismatch repair; **pol** DNA polymerase; **TetR** tetracycline-resistant

Introduction

'Adaptive' and 'stationary-phase' mutation are terms used to describe a collection of stress responses in which cells exposed to non-lethal stresses respond by promoting mutations. Some of these stationary-phase mutations may confer an advantage in the growth-limiting environment, and so are called 'adaptive' mutations, whereas as others confer no special advantage. The existence of stress-promoted mutation mechanisms implies that evolution may be hastened during stress [reviewed by **1]. Stationary-phase mutations have been reported in several different bacterial and yeast assay systems, under various kinds of stress, and occurring by various mutation mechanisms including transposon-mediated insertions and deletions, substitution and frameshift (*i.e.*, point) mutations and gene amplification. Thus there is no universal stationary-phase mutation mechanism, but rather, at least a few distinct ones that may be specific to the particular kinds of environmental stress applied, the genotype of the cells assayed, or both. Here we review recent advances from studies of a *lac* frameshift reversion assay system in *Escherichia coli* [2] in which a recombination protein-dependent stationary-phase mutation mechanism occurs [see Ref. **1 for a recent comprehensive review]: the documentation of adaptive gene amplification [**3]; the demonstration that chromosomal (not just plasmid-borne) genes undergo recombination-dependent mutation [**4]; and the discovery that a special error-prone DNA polymerase, pol IV or DinB, is required for

adaptive point mutation in this system [**5]. See [**1,*6] for reviews of this and other adaptive mutation mechanisms. The Lac system represents a mechanism of inducible genetic change under stress that uses homologous recombination proteins and a special mutator DNA polymerase. We consider programmed genetic change responses employed by prokaryotic and eukaryotic pathogens and suggest that some of these may work similarly, using mutator DNA polymerases of the DinB/UmuDC superfamily.

Stationary-phase point mutation in the Lac system

In the Lac frameshift reversion assay [2], *E. coli* cells deleted for their chromosomal *lac* (lactose catabolism) genes and harboring an F′ conjugative plasmid carrying a *lac* +1 frameshift allele are spread onto solid lactose minimal medium on which they cannot grow. Any Lac^+ mutants formed during growth of the cultures before plating on lactose appear as colonies in about two days. Additional Lac^+ colonies accumulate over the next week, and result from stationary-phase mutation mechanisms that occur after exposure to the lactose medium [reviewed Ref. **1] by two distinct mechanisms, one producing point mutations and one gene amplifications.

Recombination-dependent stationary-phase point mutation

Most of the late (stationary-phase or adaptive) Lac^+ mutant colonies are frameshift reversions, nearly all of which are –1 deletions in small mononucleotide repeats [7,8]. By contrast, growth-dependent Lac^+ mutants are more heterogeneous [7,8]. Simple repeat deletions resemble DNA polymerase errors formed by a template slippage mechanism [reviewed by**1]. Such errors are usually corrected by the post replicative mismatch repair (MMR) system, however MMR becomes limiting during stationary-phase mutation in this system, at the level of limiting MutL protein [9,10, and see Refs. *11,*12 for further discussion]. The mechanism of stationary phase-specific MMR limitation in this system is not yet understood. Because the number of MutL molecules per cell does not decline during lactose starvation, two (non-exclusive) possibilities seem reasonable [10]. MutL levels might decline only in those cells generating mutations, which, we shall see (below), are a small subpopulation of cells. Alternatively, MutL might be titrated by excess polymerase errors, or both. The stationary-phase mutation mechanism requires homologous recombination and double-strand break-repair proteins RecA, RecBC and RuvABC [13-15] implicating both DNA double-strand breaks (DSBs) or ends (DSEs) and recombination in the process, either directly or indirectly [**1]. In direct models (Fig. 1), recombinational repair of DSEs (formed in stationary phase by any number of possible mechanisms, reviewed by [**1]) is proposed to prime DNA

replication during which polymerase errors occur leading to mutation at sites of DSBR [13-16]. Indirect models are also possible in which DSBR and mutation are not physically linked [**1]. An SOS response is required for efficient point mutation in the Lac system [2,**17]. SOS is the bacterial DNA damage repair and cell cycle checkpoint control response [reviewed by **18]. The SOS response leads to induction of *trans* acting proteins involved in recombination, repair and mutation, including the error-prone DNA polymerase, pol IV, which is required for most stationary-phase point mutation in this system [**5, discussed below].

Cell subpopulation(s)

Some or all of the point mutations in this system occur in a hypermutable subpopulation of cells (hypothesized by Hall [19]) as inferred from the high frequency of unselected mutations in other genes among Lac^+ revertants, but not among similarly starved Lac^- cells [20,**21,**22]. See [**1,**4,**21,*23,*24,*25] for discussion of whether one or more than one cell population contributes to stationary-phase point mutation in the Lac system. These data (and others [**4,26]) also demonstrate that mutations in this system are not directed preferentially to *lac* or genes near it, as was hypothesized [reviewed by**1]. The proposal that the selective environment induces transient hypermutation in a cell subpopulation has important implications for microbial populations under various

stresses, including populations occupying the various niches that a pathogenic organism must pass through to colonize a host (discussed below).

Recombination-dependent mutation in the bacterial chromosome

A long-standing issue in the Lac system is whether the distinct, recombination protein-dependent mutation mechanism operating at *lac* on the F′ is also a mechanism of general genetic change for the bacterial chromosome. On the one hand, stationary-phase Lac^+ mutation on the F′ requires the transfer (Tra) functions of the F conjugative plasmid, although not actual DNA transfer [27-29]. Also, one *E. coli* [28] and one Salmonella [27] chromosomal site did not undergo RecA-dependent mutation in stationary phase, in F^- cells. Involvement of *trans* acting plasmid-encoded functions has been suggested [**22]. On the other hand, hypermutation of chromosomal sites occurs during Lac^+ stationary-phase mutation [20, **21,**22,*23], and does so with an uneven, hot and cold site distribution; one gene (*upp*) acquires10 times more loss-of-function mutations than the entire maltose (Mal) or xylose (Xyl) fermentation regulons (> 7 genes for Mal) [20]. A key question is whether those chromosomal mutations occur *via* a mechanism similar to the one generating Lac^+ mutations on the F′. Recent work shows that they do. Measuring frameshift reversion in a chromosomal tetracycline-resistance (*tet*) gene engineered into the chromosomal *upp* site in cells carrying the F′, Bull and colleagues

find that chromosomal TetR mutations increase during exposure to lactose medium in a RecA- and RuvC-dependent manner [**4]. The SOS mutator DNA polymerase, pol IV, required specifically for stationary-phase mutation at *lac* [**5] is also required for chromosomal TetR mutation [**4]. Thus recombination protein- and pol IV-dependent mutation is not limited to plasmid borne sites. Whether *trans* acting functions of the F′ are required has not been determined. Because most wild bacteria carry conjugative plasmids (and about 15% of *E. coli* and Salmonella carry F-homologous plasmids [30,31]) this stationary-phase mutation mechanism is likely to pertain to many different bacteria, regardless of whether conjugative plasmid functions are found to be required for mutation at chromosomal sites.

Adaptive amplification

Last year, a second mechanism of stationary-phase genomic change was identified using the Lac assay [**3]. The *lac* +1 frameshift allele produces a small amount of β-galactosidase (1-2 % of that of the wild-type gene). Amplification of this allele to 30-50 copies produces enough β-galactosidase to allow growth without acquisition of a compensatory frameshift mutation. Amplification was shown to be adaptive, that is, formed in response to the lactose selective medium [**3], and is a reversible genetic change that allows escape from the stress of starvation. Amplification had been

suggested to be an intermediate leading to point mutation in the Lac system [16]. However, the recent study shows that amplification and point mutation are parallel pathways—amplified DNA does not lead readily to point mutation in this system [**3]. The amplification and point mutation pathways are further distinguished in that whereas adaptive point mutation requires an SOS response [2,**17] and SOS-controlled DNA pol IV [**5], adaptive amplification requires neither [**5]. Figure 2 illustrates a scheme for the mechanisms of the parallel adaptive point mutation and amplification mechanisms in the Lac system.

Mutator DNA polymerases of the DinB/UmuDC superfamily

The study of mutation has been energized by the discovery that many organisms encode error-prone DNA polymerases of the newly found DinB/UmuC/Rad30/Rev1 superfamily [reviewed by **32,**33]. This superfamily includes members in prokaryotes, eukaryotes, and archaea. Knowledge of its existence has increased the number of DNA polymerases known in *E. coli* from three to five, and has added four new DNA polymerases to those known in humans, prompting questions as to their function(s). Some of these polymerases err two orders of magnitude more frequently than normal replicative polymerases [*e.g.*, **34,**35,*36,*37,*38,**39,*40,*41]. Many function in DNA damage tolerance or repair. For example, the human tumor suppressor protein

XPV (encoded by *RAD30a*) [reviewed **32,**33] and $UmuD_2'C$ (pol V) of *E. coli* [**35;**39] are translesion DNA polymerases. These polymerases insert bases opposite sites of DNA base damage that otherwise block replication, and so can allow damage tolerance when repair has been incomplete. Most of these polymerases examined *in vitro* make errors on lesion-containing and undamaged DNA templates. Many are thought to make mutations *in vivo* as misincorporation errors in translesion synthesis opposite damaged bases or abasic sites [**42]. However, not all of these polymerases have known lesion bypass activity. The error-prone nature of these polymerases has led to proposals [*e.g.* , Refs. **32,*43] of roles in mutational processes under cellular control, such as somatic hypermutation within immunoglobulin genes, in which two DinB/UmuDC superfamily polymerases and also the *REV3*-encoded error-prone polymerase have now been implicated [reviewed by **44].

DinB/pol IV and its role in mutation

The *dinB* gene of *E. coli*, encoding pol IV was discovered in a screen for damage-inducible (*din*) genes that are overexpressed as part of the SOS response [45]. The gene was cloned later under the name *dinP*, and although the *dinB* designation has precedence [46], *dinP* is used commonly in sequence annotation of genomes. Phenotypes associated with *dinB* mutations or overexpression suggest a role in mutation in undamaged DNA.

First, cells carrying an insertion in *dinB* are defective in phage λ untargeted mutagenesis [reviewed by **18,**32,**33], in which, *E. coli* cells irradiated with UV light are infected with phage λ, that then experience10-100 fold higher mutation than phages infecting non-irradiated hosts. Because the phage are not irradiated, this suggested that pol IV increases mutation in undamaged DNA. Second, over-production of pol IV *in vivo* leads to a 4- to 800-fold increase in mutation in the absence of DNA damaging agents [46,*47]. Both substitution and frameshift mutations are elevated, with frameshifts at mononucleotide repeats increased 100-800 fold. Purified pol IV enzyme is an error prone DNA polymerase [**34] and makes both frameshift mutations and substitutions on undamaged DNA templates. Pol IV is not capable of translesion synthesis across typical damaged bases *in vitro*. Thus, it is possible that pol IV is not a translesion polymerase, and that mutations attributed to pol IV *in vivo* may result from synthesis on undamaged template DNA. *In vivo* work apparently contradicting this idea is unfortunately difficult to interpret: *dinB* is the first gene in an apparent four gene operon in *E. coli* [see Ref.**5 and references therein]. Studies suggesting a loss of translesion mutation *in vivo* in cells deleted for *dinB* [*48] and part of the next gene downstream [see Ref.**5] are thus not yet definitive regarding a role for pol IV in mutation opposite lesions.

What function does pol IV serve in *E. coli*? Recent work on stationary-phase mutation in the Lac system indicates that one function of pol IV is promoting mutations in the *E. coli* genome under stress. Pol IV is required for recombination-dependent stationary-phase mutation both at *lac* on the F′ [**5], and at the chromosomal *upp*::*tet* site of Bull *et al.* [**4]. Pol IV is required specifically for mutation in stationary phase, and not in growing cells [see Ref.**5, for reference to discussion of an apparently contradictory report]. These results, generated with a non-polar *dinB* allele, allow unambiguous assignment of a role for pol IV in stress-inducible mutation. By extension, other members of the DinB family that are present in other organisms, and whose functions are not yet known, may play similar roles. Two other DNA polymerases are induced during an SOS response: the well characterized error-prone lesion bypass polymerase pol V (UmuD'C) and the error free pol II. Neither of these is required for stationary-phase mutation in the Lac system [2,**17, and references cited in Ref.**1].

Over two decades ago, Radman and Echols suggested that the SOS response might include inducible mutation enzymes, hastening evolution during dire circumstances in which genetic stasis is disadvantageous [49,50]. Both pol IV and pol V might play such roles. Mutation promotion could be an important function of these enzymes regardless of whether the polymerase also functions in DNA damage tolerance or repair—which, after

all, become necessary during stress. Pol IV may be a mutation enzyme, working to generate mutation in undamaged DNA or at a type of endogenous damage yet to be determined.

Antigenic variation - *Trypanosoma brucei*

Antigenic variation refers to a collection of processes by which pathogenic microbes change their surface antigens to avoid detection by the host immune response [reviewed by 51]. Surface antigens subject to antigenic variation include porins, pili, fimbriae and other surface molecules. Antigenic variation mechanisms fall into several broad categories: recombinational, mutational and transcriptional.

The eukaryotic pathogen *Trypanosoma brucei* appears to use all three mechanisms of antigenic variation [reviewed in 52,53] for the expression of variant surface glycoproteins (VSGs). *T. brucei* belongs to the family of African trypanosomes that cause sleeping sickness, and contains about 1000 VSG genes in its genome, of which only one is expressed at a time [54]. Change from expression of one VSG to another occurs with variable frequencies, between 10^{-2} to 10^{-6} / cell / generation [see references in 51]. VSG expression is thought to occur from only one of about 20 telomere-linked sites in the genome [55,56]. The remainder are transcriptionally silenced [57], resulting in a system

in which VSG expression can be accomplished in a number of ways. A silent VSG copy can be recombined into an expression site [58], or altered transcription patterns in the cell can lead to VSG transcripts from alternative telomere-linked copies [57].

Mutation-mediated antigenic variation in this system is apparent from inspection of recombinants after silent VSGs are moved into the transcriptional active site. As many as 1 nucleotide in 100 are mutated in the newly recombined VSG (these changes are not present in the silent copy) [58,59]. The expressed copy of a VSG appears to be mutated during recombination into the transcription active site, generating new epitopes without jeopardizing the parent gene from the genome.

There are some interesting commonalties between antigenic variation in *T. brucei* and stationary-phase mutation in the *E. coli* Lac system. Stationary-phase mutation requires the homologous recombination protein RecA [2,13], and VSG antigenic variation is dependent upon the eukaryotic RecA homologue Rad51 [60]. Cells that undergo stationary-phase mutation have hot and cold sites for mutation [reviewed Ref.**1] and in *T. brucei* mutations appear to occur only within the open reading frame of the newly expressed VSG [58]. This led the authors to postulate that mutation occurs *via* an RNA intermediate and a sloppy reverse transcriptases expressed from one of the many

retrotransposons found in *T. brucei*. We propose an alternative model in which homologous recombination primes DNA synthesis involving an error-prone DNA polymerase, and these errors persist as mutations (as shown in Figure 1). In support of this model and its proposed similarity to the Lac^+ point mutation mechanism, *T. brucei* has at least three homologues in the DinB/UmuC/Rad30/Rev1 family (a DinB, a Rad30 and a Rev1 homologue) (Table 1).

Antigenic variation - prokaryotes

Mutation plays a slightly different role in antigenic variation in prokaryotes. Many prokaryotic pathogens use mutation as a regulatory tool, to turn on and off expression of various different surface protein genes [61]. Typically, these genes have a simple nucleotide repeat tract within the promoter region (transcriptional control), or in early regions of the open reading frame (translational control). Changes in the length of the tract result in the promoter being on or off (for transcriptional mechanisms), or result in either shortened or full-length protein being produced (for translational mechanisms). For example, in *Mycoplasma fermentans* transcription of P78 (part of an ABC transporter) requires the presence of a tract of 7 adenines in the mRNA [62]. Deletion of a single adenine results in loss of expression of P78, and loss of that particular surface antigen.

Genera described as using strand-slippage regulatory mechanisms include *Bordetella* [63], *Campylobacter* [64], *Haemophilus* [65], *Mycoplasma* [62,66], and *Neisseria* [67]. Antigenic variation in these cases typically occurs at rates of 10^{-2} to 10^{-5} /cell/generation. The mechanism of these antigenic variation events is largely unexplored, but Moxon *et al.* [61] suggest that these are regulated. We suggest that DNA pol IV and its homologues are candidates for involvement in mutational antigenic variation. Regulation of antigenic variation could be accomplished by increased expression of DNA pol IV during times of stress. Stress (for example oxidative) could be caused in the context of an immune response. If this were the case, then the resulting mutations would be adaptive in the same sense that Lac^+ mutations are in the *E. coli* system. In BLAST searches of partly completed microbial genomes, we find that most prokaryotes carry homologues of the DinB/UmuC/Rad30/Rev1 superfamily, including all (but *Haemophilus*) of the genera listed above (Table 1). For example, *Bordetella* and *Neisseria* carry homologues of DinB that have at least 45% sequence identity and 63% sequence similarity to DinB. This model predicts that mutations affecting *dinB* homologues will prevent or decrease mutational antigenic variation.

Mutation in pathogens in general

In several systems, heritable mutator mutants (notably, cells defective in MMR, with mutation rates orders of magnitude higher than wild-type) make up a small proportion of the population of cells in a chronic infection [68-**71]. This suggests that a high mutation rate is beneficial, providing new adaptations to the stressful environment of a host. However, less than 10 % of the population infecting a host are mutator mutants. This suggests either that the benefit of being a mutator is a transient one, and regaining a wild-type MMR gene is required for long-term success of a population [**72,**73] or that many cells in these populations undergo periods of transiently high mutation rate without heritable loss of repair protein genes [reviewed in **1]. We prefer the idea that both transient and heritable mutator phenotypes contribute to the long-term survivability and evolvability of microbial species. Mutability may be a characteristic selected in pathogens as they pass through severe bottlenecks in population size, and must generate diversity *de novo* each time they infect a host [*74]. We suggest that induction of mutator DNA polymerases could produce a transient mutator state both directly by excess errors, and also by those errors titrating MMR [10,**5] thereby producing transient MMR-deficiency without loss of MMR genes. This might account for the many successfully adapted pathogens that have not lost MMR genes. Transient mutability

would be a survival mechanism without the long-term costs of mutability incurred after adaptation to the stress [**75].

Antibiotic resistance also contributes to pathogenesis and can be acquired by mutational mechanisms [**76] that might be inducible by stressful environments including those selecting resistance [77, **78]. Even lethal antibiotics cause non-lethal stress at lower concentrations that must occur frequently in patients and nature [**76]. Transient hypermutation like that in the Lac system has been suggested as a basis for multiple drug resistance in *Mycobacterium* [**78].

Conclusions

So far, the only roles demonstrated for the prototype of the DinB family, pol IV of *E. coli*, are in the induction of mutation on apparently undamaged DNA. Whether or not it also functions in DNA damage repair or tolerance, the 'mutator' aspect of pol IV function leads us to propose that it and its homologues might be important in circumstances in which mutations are beneficial. In microbial pathogens such circumstances could include antigentic variation, antibiotic resistance, and generally hastened evolution *via* transient mutator induction by titration of MMR proteins. During these circumstances, mutability may be a programmed response, as it appears to be in stationary-phase mutation.

Investigation of phenotypes of cells lacking pol IV homologues may support this hypothesis. We look forward to better understanding of the functions of DinB homologues in microbial pathogens.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest

** of outstanding interest

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A recent comprehensive review of adaptive mutation in the *E. coli* Lac system and other bacterial and yeast adaptive mutations mechanisms. Rigorous experimental definitions of 'adaptiveness' are discussed, an indirect model for the role of recombination proteins in stationary-phase mutations is considered, as are functions of eukaryotic DinB/UmuDC superfamily polymerases and programmed mutation in eukaryotes.

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Rigorous evidence is presented that gene amplification in *E. coli* can be an adaptive response, induced by conditions that select the amplified DNA. Also, a model in which

adaptive Lac$^+$ mutations were postulated to be standard growth-dependent mutations accumulated in multiple copies of amplified DNA [Ref. 16] is tested and fails tests of three of its predictions. Amplification does not appear to be a precursor to adaptive Lac$^+$ point mutation in the *E. coli* system.

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Table 1. Some pathogenic (and other) microbes carrying DinB/UmuC superfamily homologues

Major taxonomic division	Genus	Major taxonomic division	Genus
Prokaryotes		Proteobacteria (cont.)	*Actinobacillus*[b]
Firmicutes		γ subdivision	*Escherichia*
Bacillaceae	*Bacillus*[a,b]		*Klebsiella*[h]
	Staphylococcus		*Legionella*[i]
	Mycoplasma		*Pasteurella*
	Ureaplasma		*Pseudomona*[a]
Clostridiaceae	*Clostridium*[c,d]		*Salmonella*[d,h,j]
	Enterococcus[a]		*Shewanella*[a]
	Lactococcus[e]		*Vibrio*
	Streptococcus[a,b,d]		*Yersinia*[d]
	Corynebacterium[d]		*Geobacter*[a]
	Mycobacterium[a]		*Desulfovibrio*[a]
Proteobacteria		Spirochaetales	*Treponema*[a,k] (but not
α subdivision	*Caulobacter*		*T.pallidum*)
	Mesorhizobium[f]	Green non-sulfur	*Dehalococcoides*[a]
	Sinorhizobium[g]	bacteria	
		Eukaryotes	*Candida*[d]
			Saccharomyces
			Schizosaccharomyces[d]
			Plasmodium[a,d,g]
			Trypanosoma[a]

β subdivision	*Bordetella*[d]		
	Burkholderia[d]		
	Neisseria[b]		
		Archaea	*Halobacterium*[l]
			Sulfolobus

This table summarizes the results of a non-exhaustive BLAST search [79] for *dinB* homologues in some pathogenic and other microbes. One or more species of genera listed possess sequences with at least 25% sequence identity/ 42% sequence similarity to the *E. coli dinB* gene over a length of ≥ 68 amino acids. The similarity cutoff was an e-value of 1×10^{-8} (indicating a probability of a sequence of that degree of similarity appearing in the database by random chance as being 1×10^{-8}). This search does not discriminate between branches of the DinB/UmuDC/Rad30/Rev1 superfamily of DNA polymerases. A more detailed summary of the results of this search, including references to the published sequence data used, is posted at http://www.imgen.bcm.tmc.edu/rosenberg/mchDinB-UmuC_table.html. Unpublished preliminary sequence data were obtained from sequences deposited in the NCBI Unfinished Genomes website by the following organizations (a) The Institute for Genomic Research website at http://www.tigr.org; (b) The University of Oklahoma's Advanced Center for Genome Technology http://www.genome.ou.edu/; (c) The Genome Therapeutics Corporation http://www.cric.com/; (d) The Sanger Centre

http://www.sanger.ac.uk/; (e) INRA, Genetique Microbienne http://www.inra.fr/; (f) Kazusa DNA Research Institute http://www.kazusa.or.jp/en/; (g) Stanford Genome Technology Center http://www-sequence.stanford.edu/; (h) Genome Sequencing Center in Washington University in St. Louis http://genome.wustl.edu/gsc/; (i) Columbia Genome Center http://genome3.cpmc.columbia.edu/~legion/; (j) University of Illinois Urbana Champaign http://www.salmonella.org/; (k) University of Texas Health Sciences Center http://www-mmg.med.uth.tmc.edu/sphaeroides/; (l) Institute for Systems Biology http://www.systemsbiology.org.

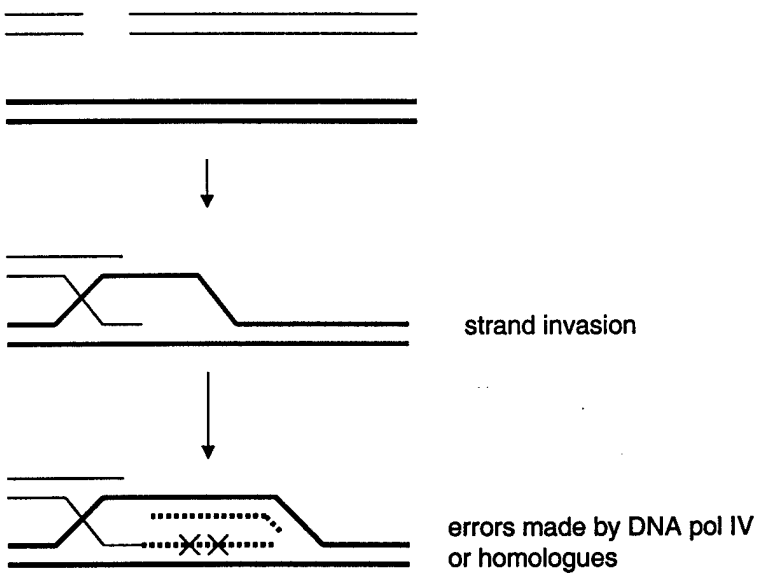
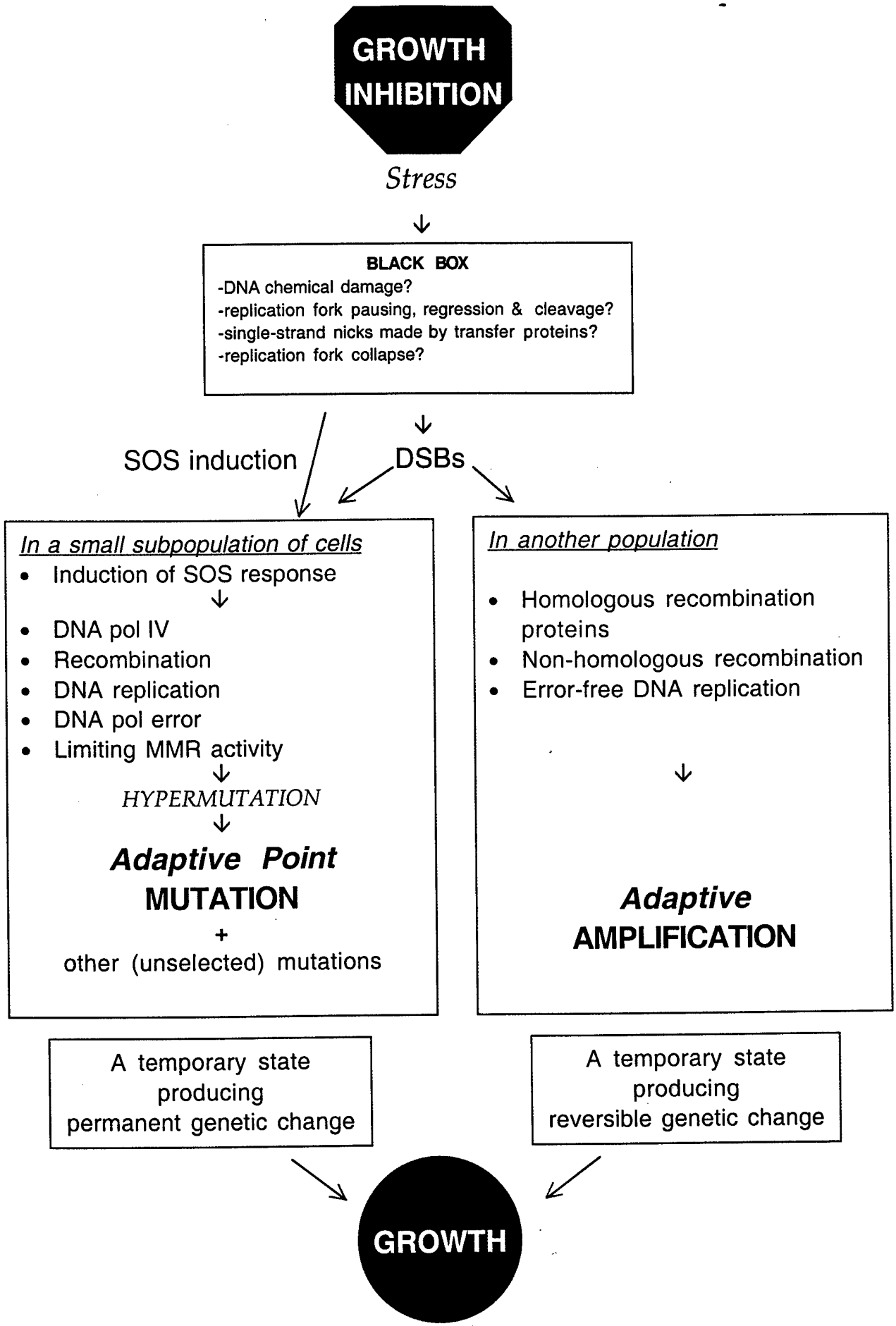


Figure 1: A model for both stationary-phase mutation and mutational antigenic variation seen in *T. brucei.* See text. Strand invasion of a homologous region of DNA primes DNA synthesis. Errors made in this region by error-prone DNA polymerases persist as mutations. Possible sources of homology in stationary-phase bacteria are sister molecules, gene duplications, and DNA taken from the environment [reviewed Ref. **1].

Figure 2: Model for mechanisms of recombination-dependent adaptive point mutation and amplification in the *E. coli* Lac frameshift reversion assay system. See Ref. [**1] for a review of data leading to this scheme, and of the possible mechanisms by which DSBs may be generated in the F′ and chromosomal DNA. The point mutation response is described in the text. For adaptive amplification, the amplified DNA is present as direct repeats the unique junctions of which have been mapped to regions of non-homologous joints [**3], as observed previously in bacterial amplification [reviewed by **3]. This suggests at least one initial non-homologous recombination event [**1,**3] although the dependence of total late Lac^+ colonies on homologous recombination proteins implicates Rec protein involvement in the adaptive amplification response as well. Perhaps Rec proteins process DSEs that engage in non-homologous recombination leading to amplification [**1]. Amplification does not require an SOS response or pol IV [**5] and the amplified isolates are not hypermutated as Lac^+ point mutants are [**3]. Thus the two appear to arise from different subpopulations of the starving cells.


GROWTH INHIBITION
Stress
BLACK BOX
-DNA chemical damage?
-replication fork pausing, regression & cleavage?
-single-strand nicks made by transfer proteins?
-replication fork collapse?
SOS induction
DSBs
In a small subpopulation of cells
• Induction of SOS response
• DNA pol IV
• Recombination
• DNA replication
• DNA pol error
• Limiting MMR activity
HYPERMUTATION
Adaptive Point MUTATION
+
other (unselected) mutations
In another population
• Homologous recombination proteins
• Non-homologous recombination
• Error-free DNA replication
Adaptive AMPLIFICATION
A temporary state producing permanent genetic change
A temporary state producing reversible genetic change
GROWTH